DIACYLGLYCEROLS
Healthy Fats of the Future?

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Professor Dr. Lai Oi Ming

# INAUGURAL LECTURE series

## Prof. Dr. Lai Oi Ming

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PROFESSOR DR. LAI OI MING

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BSc (Hons) (UKM), MSc (UKM), PhD (UPM)

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## ABSTRACT

Malaysia is the fattest nation in South East Asia. A recent survey by Social Security Organization (SOCSO) Malaysia on 308,039 Malaysian employees in 2013 showed that 36.94% were overweight, 17.63% were obese, 13.14% had hypertension, 61.76% had hypercholesterolemia and 8.45% had diabetes (The Star, 27 Feb 2015). Malaysians' present lifestyle and culture were among the reasons cited for the rise in these figures. Excessive intake of fat in the diet has been linked to diseases such as heart disease, cancer, obesity and possibly, gallbladder disease. Increased saturated fat intake is associated with high blood cholesterol and increased risk of coronary heart disease. It is difficult for individuals to change their dietary habits to reduce or minimize fat intake, while enjoying their favourite foods. This problem and the interest shown by consumers in structured lipids led to the search, by the food industry and scientific community, for "Healthy Fats of the Future". Just as micronutrients have been lauded to prevent disease, so too has the effect of structured lipids like diacylglycerols (DAG) on obesity and weight-related disorders. The physiological effect of DAG is believed to be attributable to its metabolic pathway, which is different from the normal triacylglycerol metabolism. With structured lipids, we can combine the different positive and nutritionally valuable fatty acids in palm oil to produce a potent structured lipid with maximum benefits and minimum adverse effects. The main aim of this talk is to provide a comprehensive review of palm-based DAG with emphasis on the production, process developments, applications and animal trials.

## INTRODUCTION

Generally, dietary fats and oils are classified as essential macronutrients which are prerequisites to maintaining the physiological functions of a human body (Skeaff and Mann, 2012). Being the most energy sufficient source of food, supplying as much as 9 calories energy, it provides us with the basic energy for daily activities. It also serves as the building blocks of healthy cells, carriers for fat-soluble vitamins, organ protector and body insulator. Dietary fats and oils also play an important role in enhancing the sensory and textural properties of food products (Drewnowski, 1997). The presence of dietary fats and oils indirectly improve the palatability, mouthfeel, aroma, flavour and glossiness of food. Often, fat-dense foods, such as deep fried and bakery products, are the ones perceived to be more tasty to the consumers. However, increased intake of these high-fat foods coupled with inadequate physical activities may lead to overwhelming incidences of obesity and heart diseases (Golay and Bobbioni, 1997). A recently published research report also indicates that the prevalence of obesity is rather severe in Malaysia as compared to other Asian countries with 45.3% of its population being overweight and the obese population forecasted to skyrocket in the next decade (Ng *et al*. 2014). Obesity is basically due to excessive fat accumulation in the body which poses a risk to human health. Various research studies strongly associate obesity with life-threatening diseases, such as heart disease, cancer, diabetes mellitus and hypertension, besides mental trauma and physical discomfort (Lavie *et al.*, 2011, Lavie *et al.*, 2009, Misra *et al.*, 2010). The above phenomenon has certainly prompted consumer awareness in reducing the consumption of high fats diets and to some extent triggered the mandatory governmental legislations to control the intake of unhealthful fats such as *trans* fat and saturated fats (Brownell and Pomeranz, 2014, Downs *et al.*, 2013). This

subsequently led to the search and discovery of better and healthy dietary fats and oils or the so-called "functional oil/structured lipid" (Osborn and Akoh, 2002). The term "functional oil or structured lipid" refers to dietary fats and oils that have the potential to promote health and reduce the risk of disease (Alabdulkarim *et al.*, 2012). The concept of structured lipids was first introduced by Babayan (1987), for nutritional and medical purposes. At that time, Babayan came out with a structured lipid called medium chain triglyceride (MCT) that possessed the ability to be metabolised rapidly compared to the conventional fats and oils. It was utilized in the area of malabsorption cases particularly for patients that were critically ill as well as an instantaneous energy drink for athletes (Babayan, 1987). Thus, sometimes, the structured lipid can also be referred to as "nutraceuticals" or "pharmafoods". With the advancement of science and technology, various types of structured lipids which serve different purposes have been produced commercially (Kirk *et al.*, 2008). Table 1 summarizes the list of various types of commercially available structured lipids together with their respective methods of synthesis and applications.

**Table 1** List of various types of commercially available structured lipids and their respective methods of synthesis and application

| Structured lipid | Company | Method of Synthesis | Application |
|---|---|---|---|
| Betapol | Loders Croklaan B.V. (The Netherlands) | OPO type fat sn-2 palmitic | Human milk fat substitute |
| CAPRENIN | Procter and Gambler | Esterification of behenic acid, capric and caprylic with glycerol | Low calories synthetic fat |
| Behenin | Fiji Oil Corporation Limited | Interesterification of trioleic and behenic acid | Anti-bloom in chocolate |
| Captex (MCT) | ABITEC Corporation | Esterification of caprylic and capric acid with glycerol | Low calories synthetic fat |
| DAG | Kao Corporation | Glycerolysis between glycerol and triglyceride | Low calories synthetic fat |
| MLCT | Nisshin Oillio Limited | Interesterification of MCT with long chain triglyceride | Low calories synthetic fat |
| SALATRIM | Nabisco | Interesterification of short triglyceride (triacetin or butyrin) with long chain triglyceride | Low calories synthetic fat |
| Structolipid | Pharmacia AB | Interesterification of MCFA and LCFA | Total parental nutrition |

(MCT = Medium chain triglyceride; DAG=diacylglycerols; MLCT = medium- and long-chain triacylglycerols, MCFA = medium chain fatty acids; LCFA = long chain fatty acids)

In this 21st century, synthesis of the above mentioned and other new types of structured lipids is made possible either with the aid of enzyme lipases or chemical catalysts whereby these catalysts are responsible for modifying the structure of the native fats and oils to produce lipids with the desired functionalities (Iwasaki and Yamane, 2000). When a chemical catalyst is used, the reaction is more randomized in order thereby producing products that are less pure. However, chemical catalysts incur lower production costs compared to enzymes. Meanwhile, utilizing enzymes in fats and oils modification has several beneficial effects. Enzyme catalyzed reaction is more selective, specific, mild and environmentally friendly. However, enzymes can be costly and more easily susceptible to inactivation under harsh reaction conditions. Nevertheless, with the development of various immobilization techniques, stable enzymes that can be used repeatedly without losing their activity have been developed, which can reduce the cost of production of structured lipids (Bickerstaff, 1997).

The enzyme or chemical catalyzed lipid modification reactions can be categorized into interesterification, esterification, alcoholysis, acidolysis, hydrolysis and aminolysis, as shown in Figure 1, depending on the type of substrate used for the reaction (Gandhi, 1997). The modifications are made possible by placing the desired fatty acid at a particular position in a triacylglycerol molecule so that the fatty acids can be sent directly to the body for nutritive and therapeutic purposes to combat certain diseases and metabolic conditions, due to the improvement in the rate of digestion, adsorption, and metabolism (Lee and Akoh, 1998).

Ultimately, lipid modification also results in the alterations either in fatty acid composition or fatty acid position, which indirectly affect the physicochemical characteristics of the triacylglycerol, like crystallization and melting points and solid fat content (Lee et al.,

2008; Mu and Porsgaard, 2005). Hence, at times, modification of lipids is conducted to change the physicochemical characteristics of conventional fats and oils in order to expand their application in various food products. As long as substitution of structured lipids does not affect the flavor and the safety of the food, structured lipids can not only be utilized for food applications but also for medical and nutritional purposes.

## DIACYLGLYCEROLS – STRUCTURE AND PROPERTIES

The structure of conventional oils or the so-called triacylglycerols, where the glycerol backbone is attached to three fatty acids (FA), is different from the ester of glycerol in diacylglycerols (DAG), where the glycerol backbone is esterified with two FA. The two FA in DAG can be esterified in different positions leading to the existence of three stereochemical forms of DAG, namely, *sn*-1,2-DAG, *sn*-1,3-DAG, and *sn*-2,3-DAG (Lo *et al.*, 2008). Figure 2 shows the structures of triacylglycerols and various isoforms of DAG.

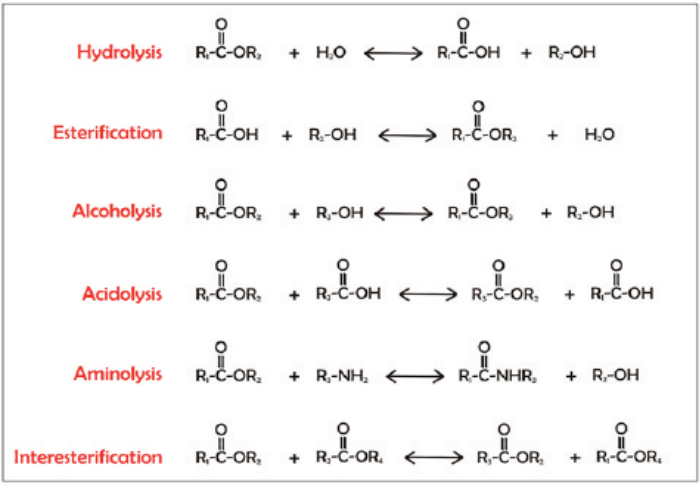


**Figure 1** Reactions catalysed by lipase

(Source: Borrelli and Trono, 2015)

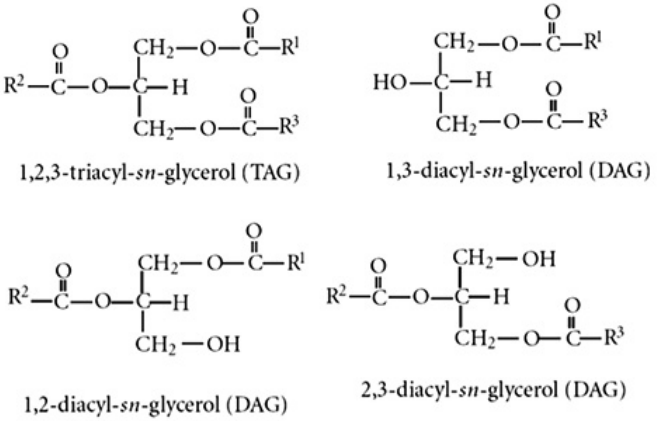


**Figure 2** Structures of triacylglycerols (TAG) and various isoforms of diacylglycerol (DAG) wherein R1, R2 and R3 are hydrocarbon chains of fatty acids esterified on the glycerol backbone**.**

**(**Source: Madawala *et al.*, 2011)

These DAG isomers will undergo acyl migration to form equilibrium at 30% - 40% of 1,2-(2,3-) DAG and 60%-70% of 1,3-DAG when "triggered" by the addition of an acid, alkali or heat (Serdarevich, 1967; Takano and Itabashi, 2002). It can be seen that 1,3-DAG is more thermodynamically stable compared to 1,2-(2,3)-DAG, mainly due to the steric effect of the molecule.

DAG is present in plant oils. It can be found naturally in various edible oils at a level of around 2% to 10% (w/w) (Holčapek *et al.*, 2003). The relative content of DAG and other acylglycerols in various edible oils is shown in Table 2. The DAG content can be as low as 0.8% in rapeseed oil and as high as 10% in cotton seed oil. Nevertheless, these values may vary depending on the variety of the oil plant, processing parameters and storage conditions. However, around 70% of the DAG that is present in vegetable oils appears in the 1,3 isoform. Besides plants, DAG can also be produced endogenously in the human body as a product of triglyceride metabolism.

**Table 2** Acylglycerols content in edible oils of various origins

| Acylglycerol | Soybean | Cotton seed | Palm | Corn | Sunflower | Olive | Rapeseed |
|---|---|---|---|---|---|---|---|
| Monoacylglycerol | - | 0.2 | - | - | - | 0.2 | 0.1 |
| Diacylglycerol | 1.0 | 9.5 | 5.8 | 2.8 | 2.1 | 5.5 | 0.8 |
| Triacylglycerol | 97.9 | 87.0 | 93.1 | 95.8 | 96.0 | 93.3 | 96.8 |
| Others | 1.1 | 3.3 | 1.1 | 1.4 | 1.9 | 2.3 | 2.3 |

(Source: Matsuo, 2004)

DAG oil has several healthful benefits and is classified as a type of structured lipid. The main nutritional functions of DAG oil vs TAG oil are suppressive effects on postprandial serum triglyceride and body fat accumulation (Maki *et al.*, 2002; Nagao *et al.*, 2000; Takase *et al.*, 2005). Most pre-clinical and clinical studies focus on the use of DAG oil as an adjunctive therapy for managing body weight through weight loss *via* reduction of body fat accumulation as well as lowering the postprandial triglyceride level, especially among the high body mass index population (Maki *et al*., 2002). However, the intake of DAG has to be controlled wisely as when weight loss happens in normal weight individuals after consumption of DAG oil.

According to Flickinger and Matsuo (2003) DAG oil can only "express" its beneficial effects at a concentration of at least 40% (w/w) which is far greater than the DAG oil found in vegetable oil. However, the high concentration of DAG oil can be enzymatically or chemically synthesized. In 1999, Kao Corporation in Japan became the first to develop and patent a cooking oil containing at least 80% DAG where the ratio of 1,3-DG to 1,2 (2,3)-DG was about 7:3. Besides DAG, the oil also comprised some triacylglycerol (up to 20%), some monoacylglycerol (up to 5%) and small amounts of antioxidants and emulsifier to maintain the quality of the DAG. The DAG manufactured by KAO Corporation is generally produced from FA derived from vegetable oil and glycerol using an immobilized 1,3 specific lipase through the esterification process. The manufacturing flow can be divided into two main processes: the production of DAG *via* enzymatic esterification reaction and the refining process that is typically used in the production of edible oil. The DAG oil was a successful product of KAO Corporation, Japan. It has been sold widely in the Japanese and US markets under the brand names Econa™ and Enova™, respectively, as the leading

healthy cooking oils. In Japan, it has been marketed as "Food for Specified Health Use". To date, DAG has been given the GRAS (Generally Recognized as Safe) status by the US Food and Drug Administration. Sales of this functional edible oil accounts for 80% of premium oils which constitute around 14% of the total Japanese edible oil market worth ¥10 billion in the early 2000s (Flickinger and Matsuo,2003). The typical FA and acylglycerol composition of the KAO Corporation DAG is listed in Table 3.

**Table 3** Fatty acid and acylglycerol composition of DAG oil manufactured by KAO Corporation, Japan

| **Fatty acid composition** | **%** |
|---|---|
| Palmitic acid | 3.0-3.5 |
| Stearic acid | 1.0-1.5 |
| Oleic acid | 38-40 |
| Linoleic acid | 47-49 |
| Linolenic acid | 8-9 |
| Saturated fatty acid | 4-5 |
| Monounsaturated fatty acid | 38-40 |
| Polyunsaturated fatty acid | 55-58 |
| **Acylglycerol distribution** | |
| Monoacylglycerol | 0.4-1 |
| Diacylglycerol | 82-87 |
| Triacylglycerol | 12-18 |

(Source: Matsuo, 2004)

By virtue of its physical properties, DAG oil has special physicochemical characteristics due to the presence of a free hydroxyl group in the structure of the DAG. Hence, the strong hydrogen bonding of the hydroxyl group and fatty acid chain arrangement of the DAG isomers ultimately result in the 1,3-DAG having approximately 10 °C higher melting temperature than that of TAG, while that of 1,2-DAG is approximately 10 °C lower than that of 1,3-DAG of the same fatty acid composition (Bockish, 1998; Formo, 1979). Further, the conformation of 1,3-DAG appears in V-shaped form, while the 1,2-DAG is hairpin-shaped. The different types of molecular arrangements of the DAG isomer are associated with its polymorphic form. 1,2-DAG exhibits α- and β'-forms but has no β-form; while 1,3-DAG has no α-form, but exhibits two types of β-form; β1 and the more unstable β2 (Nakajima *et al.*, 2004)

In a study to compare the energy value and digestibility of DAG and TAG, Taguchi *et al.* (2001) found no significant differences in terms of energy value and digestibility between two groups of rats fed with DAG or TAG containing diets. The energy value of DAG is 38.9 kJ/g, while the energy value of TAG is 39.6 kJ/g. In terms of digestibility of DAG, digestibility of the rats fed with DAG and TAG were similar at 96.3 ± 0.3% and 96.3 ± 0.4%, respectively (Taguchi *et al.*, 2001).

## SAFETY AND REGULATION OF DIACYLGLYCEROL OIL

DAG oil has a long history of use where it existed in the human diet as a natural diacylglycerol in the common vegetable oils or as components in the emulsifier system. Human consumption of diacylglycerol in the U.S population is estimated to be 3g/d. Although DAGs are indirectly consumed substances, DAG oils, when used as cooking oil by itself or as a main ingredient in prepared food, are

perceived to give different exposure to consumers. Hence, oil or food products containing high concentration of DAG are required to undergo formal safety evaluation according to modern standards. Nevertheless, since DAG oil is already present in the common food in measurable amounts and has a long safe history of use, the Food and Drug Association (FDA) considers it as a candidate for GRAS, which indicates that there are no concerns regarding its safety. The initial self-affirmation as GRAS is claimed by two categories which are, home cooking oil use and use in margarine spreads. Additionally, the GRAS categories were further extended into 10 categories which include: home cooking oil, margarine spread, bakery products, dressing for salad, pizza, mayonnaise, breakfast/snack/power bars; soup/gravies, meal replacement and frozen dinner/entrees. The energy content of the DAG oil was labeled as 120 kcal/serving, which is equivalent to that of vegetable oil, and this is determined by the bomb calorimetry and direct animal measurement (Taguchi *et al*., 2001). DAG oil typically contains 0.5 g saturated fat, 8 g polyunsaturated fat and 5 g of monounsaturated fat per serving. The published studies on animals and humans which have demonstrated that DAG is likely to be delivered directly to the liver for beta-oxidation have prompted it to be labelled and advertised as "less likely to be stored as body fat". Furthermore, sufficient evidence from these studies created a second label claim of "one is able to maintain a healthy weight when using DAG oil as part of a sensible diet". Additionally, further studies support the third claim: "triglycerides in the blood stream are lowered following a meal compared to that with the use of conventional oil". Hence, in Japan, the Ministry of Health and Welfare approved DAG oil as Food for Specified Health Use (FOSHU) in 1999. In the following year, a professional association of Japanese physicians, further suggested DAG to be beneficial in improving human health. Further,

as of December 2007, DAG oil has been authorized for marketing in Japan, United States, European Union, Canada, Australia/New Zealand and Brazil. Various studies concerning the safety of DAG oil, in relation to its genotoxicity, acute toxicity, subchronic toxicity or metabolic, has been established in animals, *in vitro* and humans. The results are consistent in supporting the lack of toxicity of ingested DAG oil (Morita and Soni, 2009; Yasunaga *et al.*, 2004). No evidence of mutagenicity or genotoxicity was found *in vitro* and *in vivo* assays (Kasamatsu *et al.*, 2005). Moreover, long term studies have shown DAG oil to give no carcinogenicity at doses up to 2500mg/kg body weight/d for up to 2 years in rats and up to 2500mg/kg body weight/d for 1 year in dog subjects. Numerous clinical studies with healthy adult volunteers showed DAG oil to be well tolerated at doses as high as 45 g DAG oil/kg body weight/d or 450mg DAG oil/person/d for 100 kg persons without reported adverse effects and with outcomes similar to those seen with TAG oil. Similarly, children and individuals with adverse diabetes and uremia showed no signs of adverse effects after incorporating DAG oil into their diet (Kasamatsu *et al*., 2005).

## PRODUCTION OF DIACYLGLYCEROL OIL

In general, DAG can be produced by esterification of fatty acids and glycerol (Lo *et al.*, 2007, Lo *et al.*, 2004a, Lo *et al.*, 2004b), glycerolysis between TAG and glycerol (Yang *et al.*, 2004), partial hydrolysis of TAG in presence of water (Cheong *et al.*, 2007), interesterification between TAG and MAG (Weber and Mukherjee, 2004) or a combination of partial hydrolysis and esterification reactions (Yamada *et al.*, 2001). The aforementioned reactions can either be catalysed by inorganic chemical catalysts, such as sodium, potassium or calcium hydroxides, or biodegradable enzyme lipases. Both processes have their pros and cons. Although the chemical

approach is cheaper, the process is conducted at high temperatures (220°C-260°C) and often requires extensive purification steps to ensure desirable product quality. In contrast, the enzymatic method is more environmental friendly and requires only mild reaction conditions.

The esterification process is the most direct method of producing diacylglycerol. It should be noted that the removal of water formed during esterification is essential to push forward the esterification reaction for the formation of diacylglycerol. In the early study of DAG production, Mazur and George, (1992) employed 1,3 position specific immobilized lipase to catalyze the esterification of fatty acid anhydride with glycerol in water immiscible hydrocarbon or chlorinated hydrocarbon to produce DAG with 41% (w/w) yield. Yoon *et al.* (2004) disclosed the production of DAG by esterifying MAG with conjugated linoleic acids in the presence of lipase. Additionally, Lo *et al*. (2007) produced DAG through enzymatic esterification of: i) oleic acid (Table 4); ii) palmitic and oleic acid mixture (Table 5); and iii) stearic and oleic acid mixture (Table 6) with glycerol, in a packed bed reactor where approximately 50 - 60 % of DAG was obtained and a minimum of 15 - 25 % of TAG was produced simultaneously. Further purification process resulted in around 90% DAG. This method produces specific DAG species: 1,3(2)-diolein-enriched, 1-palmitoyl-3(2)-oleoyl glycerol- and 1-stearoyl-3(2)-oleoyl. Interestingly, the study found that the Lipozyme RM IM lipase displayed preference towards the production of oleic acid-enriched DAG as compared to palmitic acid-enriched DAG and stearic acid-DAG. To reduce the production cost of DAG, Lo *et al*. (2004a) and Lo *et al*. (2004b), in another study, carried out an enzymatic esterification of fatty acid deodorizer distillate of corn oil and soybean oil obtained from refineries with glycerol to produce DAG with 80% yield.

**Table 4** Oleic acid incorporated DAG and TAG yields and DAG composition from pilot packed-bed enzyme reactor productions

| Production No. | DAG yield (wt. %) | TAG yield (wt. %) | DAG composition (wt. %)[1] | |
|---|---|---|---|---|
| | | | 13OO | 12OO |
| 1 | 53.9 | 18.1 | 56.8 | 14.4 |
| 2 | 54.3 | 22.7 | 55.2 | 12.5 |
| 3 | 56.7 | 24.1 | 55.1 | 13.3 |
| 4 | 56.1 | 22.5 | 56.1 | 14.4 |
| 5 | 58.2 | 23.6 | 53.1 | 13.2 |
| 6 | 56.6 | 20.5 | 54.7 | 15.1 |
| 7 | 55.8 | 24.6 | 55.6 | 14.8 |
| 8 | 53.3 | 21.4 | 55.0 | 13.2 |
| 9 | 54.2 | 23.7 | 54.1 | 11.2 |
| 10 | 51.1 | 19.7 | 56.5 | 14.4 |

[1]13OO = 1,3-diolein; 12OO = 1,2-diolein (Source: Lo *et al*., 2007)

**Table 5** Palmitic and oleic acid incorporated DAG and TAG yields and DAG composition from pilot packed-bed enzyme reactor productions

| Production No. | DAG yield (wt. %) | TAG yield (wt. %) | DAG composition (wt. %)[1] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 13OO | 12OO | 13PO | 12PO | 13PP | 12PP |
| 1 | 43.8 | 19.2 | 4.8 | 11.4 | 12.7 | 5.8 | 0.2 | 0.3 |
| 2 | 47.7 | 16.9 | 5.2 | 12.5 | 14.1 | 6.3 | 0.2 | 0.3 |
| 3 | 46.8 | 14.9 | 5.1 | 12.3 | 13.9 | 6.0 | 0.2 | 0.3 |
| 4 | 47.2 | 12.8 | 5.1 | 12.4 | 13.9 | 6.2 | 0.2 | 0.3 |
| 5 | 47.2 | 13.3 | 5.1 | 12.2 | 13.7 | 6.2 | 0.2 | 0.4 |
| 6 | 46.3 | 11.0 | 5.0 | 12.1 | 13.6 | 6.1 | 0.1 | 0.3 |
| 7 | 45.2 | 12.3 | 5.0 | 11.8 | 13.4 | 5.8 | 0.2 | 0.3 |
| 8 | 46.4 | 11.7 | 5.0 | 12.2 | 13.7 | 6.1 | 0.2 | 0.3 |
| 9 | 46.9 | 13.5 | 5.1 | 12.2 | 13.6 | 6.2 | 0.2 | 0.4 |
| 10 | 47.3 | 12.4 | 5.1 | 12.4 | 13.9 | 6.2 | 0.2 | 0.3 |

[1]13OO = 1,3-diolein; 12OO = 1,2-diolein; 13PO = 1-palmitoyl-3-oleoyl glycerol; 12PO = 1-palmitoyl-2-oleoyl glycerol; 13PP = 1,3-dipalmitin; 12PP = 1,2-dipalmitin
(Source: Lo *et al*., 2007)

**Table 6** Stearic and oleic acid incorporated DAG and TAG yields and DAG composition from pilot packed-bed enzyme reactor productions

| Production No. | DAG yield (wt. %) | TAG yield (wt. %) | DAG composition (wt. %)[1] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 13OO | 12OO | 13SO | 12SO | 13SS | 12SS |
| 1 | 41.6 | 29.1 | 1.6 | 0.5 | 11.2 | 3.3 | 25.0 | 0.5 |
| 2 | 48.2 | 23.5 | 2.1 | 0.5 | 12.8 | 3.8 | 28.3 | 0.5 |
| 3 | 49.3 | 21.2 | 1.7 | 0.4 | 13.5 | 3.7 | 30.7 | 0.4 |
| 4 | 51.1 | 19.0 | 2.0 | 0.6 | 13.7 | 4.0 | 29.6 | 0.4 |
| 5 | 49.8 | 22.3 | 1.5 | 0.6 | 12.5 | 4.0 | 28.3 | 0.5 |

[1]13OO = 1,3-diolein; 12OO = 1,2-diolein; 13SO = 1-stearoyl-3-oleoyl glycerol; 12SO = 1-stearoyl-2-oleoyl glycerol; 13SS = 1,3-distearin; 12SS = 1,2-distearin (Source: Lo *et al*., 2007)

Apart from esterification, diacylglycerol can also be synthesized using the transesterification process. Toshinori *et al.* (2000) has transesterified MAG with TAG for DAG production. The processes of utilizing MAG as raw material for DAG production however, may not be industrially cost efficient as MAG is relatively expensive.

In another process, Yamada *et al*. (2001) used a combination of hydrolysis and esterification reactions for DAG production, whereby the TAG is first hydrolyzed into free fatty acids (FFA) prior to esterification of these fatty acids with glycerol.

Lai *et al*. (2005a-b, 2006a-d) further filed 5 patents in Malaysia (PI 20066218), Europe (EP1803819), United States (US2007/0148745), Japan (2007-175049) and PCT International (WO2007/075079), for the enzymatic partial hydrolysis method for diacylglycerol production from triacylglycerol. This invention involves the reaction of triacylglycerol with water and enzymes to obtain a mixture of diacylglycerol, monoacylglycerol and free fatty acids. A high purity of diacylglycerol can be obtained after the removal of the water content, monoacylglycerol, free fatty acid and residual triacylglycerol. The advantage of this process lies in its single-step hydrolytic reaction. Similar work was carried out by Cheong *et al*. (2007) and Phuah *et al*. (2012), which utilized the lipase *Rhizomucor meihei* (RMIM) catalyzed enzymatic partial hydrolysis process for palm-based diacylglycerol production. Cheong *et al*. (2007) managed to obtain approximately 32 wt % of palm-based diacylglycerol using the following reaction conditions: 50 wt % water content, 10 wt % enzyme load, 65°C of reaction temperature and 12 h of reaction time. Following the short path distillation, palm-based diacylglycerol of 90% purity can be obtained from the crude palm-based diacylglycerol.

Choo *et al.* (2006) disclosed another interesting method whereby short path distillator under vacuum of not more than 0.01 Torr, at temperature of 300 °C and below, was used for the synthesis of DAG oil from vegetable TAG oil.

Additionally, palm kernel based diacylglycerol (PKDG) was produced by Tang (2013) using the glycerolysis process in a packed bed reactor. In this glycerolysis process, the glycerol is added in a stepwise manner (5 portions, every half hour once) where the weight ratio of glycerol/oil was 1.45:10. He found that the PKDG content increased from 1.6±0.0% to 43.5% and the triacylglycerol content decreased from 98.4% to 11.5%, after 9 hours of reaction (Figure 3).

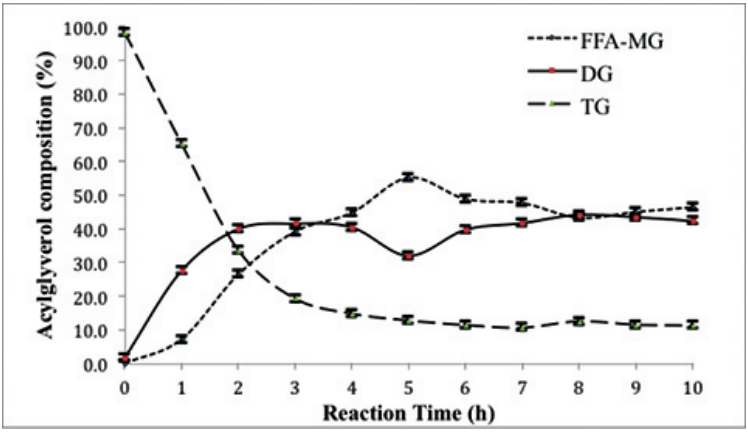


**Figure 3** Time screening for acylglycerol composition of PKDG in pilot scale reactor (FFA-MG, DG and TG). FFA-MG, free fatty acids and monoacylglycerol; DG, diacylglycerol; TG, triacylglycerol; Values are mean±SD of triplicate data.

(Source: Tang, 2013)

Although the PKDG content reached 41.6% at 3 hours, the reaction was left to proceed till 9 hours as the undesired TG content still remained high at 3 hours of reaction and this can affect the subsequent purification process. Figures 4 and 5 show the HPLC fingerprints of PKDG before and after purification, respectively. The crude PKDG produced was further subjected to short path distillation for purification to remove the unnecessary free fatty acid, monoacylglycerol and triacylglycerol in the mixture. The purification process managed to yield DAG of purity of around 90%. In another study, Sugiura *et al.* (2002) also used low temperature (0°C to 25°C) glycerolysis process to produce DAG where glycerol and TAG were the main substrates.

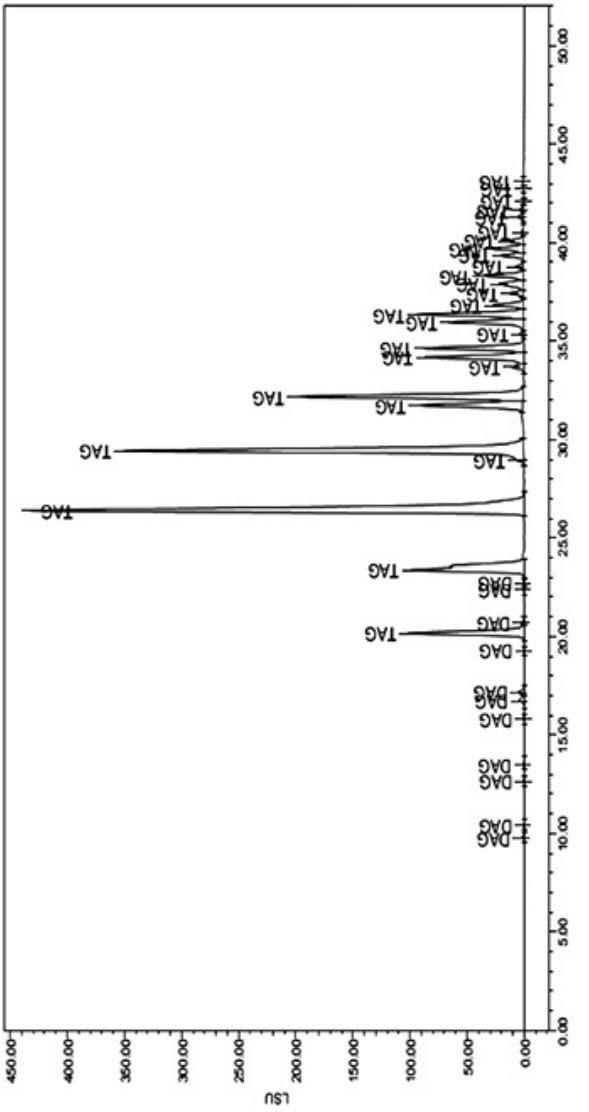


**Figure 4** HPLC fingerprint for PKTG. FFA-MAG, free fatty acids and Monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol

(Source: Tang, 2013)

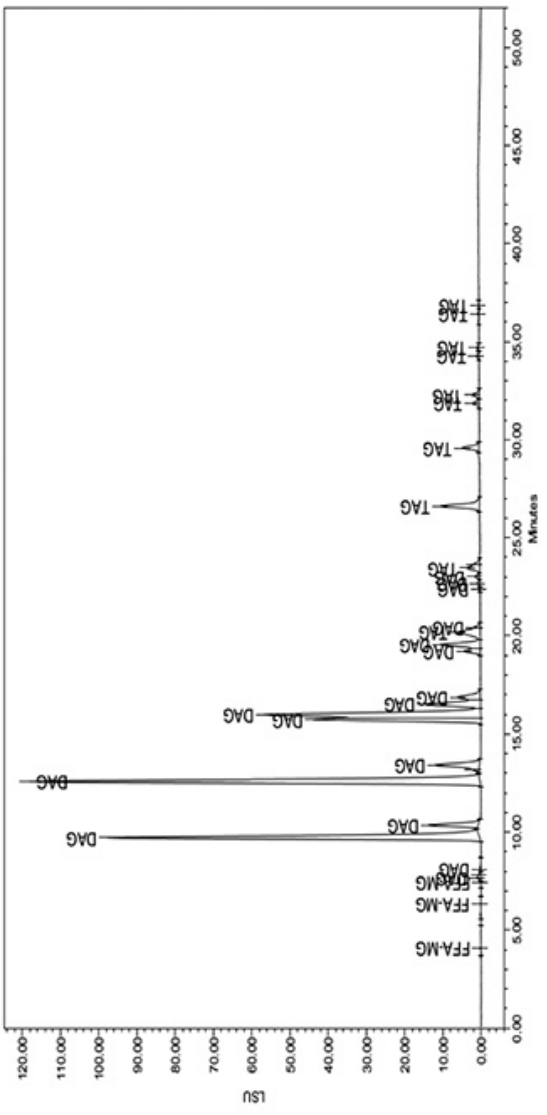


**Figure 5** HPLC fingerprint for purified PKDG. FFA-MAG, free fatty acids and monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol
(Source: Tang, 2013)

Apart from using the enzymatic approach in the synthesis of DAG, several studies have also been carried using the chemical catalyst or combination of both methods for DAG production. Utilization of resin has several beneficial outcomes. Resin is low in cost, easily separated, and can be reused for many cycles. It can be regarded as a green chemical catalyst because no toxic wastewater is generated in the process. Jacob *et al.* (2003) used similar glycerolysis approach using TAG and glycerol as the substrates for DAG production. Nonetheless, this reaction is aided by chemical catalyzed potassium acetate and requires relatively high reaction temperature of 190°C to 240°C which may incur a significant energy cost. Study on the production of 1,3(2)-diolein-enriched, 1-palmitoyl-3(2)-oleoyl glycerol- and 1-stearoyl-3(2)-oleoyl glycerol-enriched DAG using macroporous ion exchange resin-catalysed esterification of palm fatty acids with glycerol was carried out by Lo *et al*. (2007). The reaction, performed for 1.5 hr at 110°C, in the presence of 25% resin, produced around 40 wt. %, DAG and 2.5-6.0 wt. % of TAG. Various stereoisomers of DAG were obtained using oleic acid (Table 7), palmitic and oleic acid (Table 8) as well as stearic and oleic acid (Table 9) as the starting material**.** The chemical catalyst only displayed preference towards the production of oleic acid-enriched DAG. As compared to the previous study conducted by Lo *et al*. (2007), which used 1,3 specific lipase enzyme for DAG production, a slightly higher DAG yield (by 10-15 wt. %) can be obtained using Lipozyme RM IM lipase than the resin catalyst. However, the corresponding TAG yield obtained using Lipozyme RM IM was 4-5 times higher than that obtained with the resin catalyst. A patent was filed for the production of diacylglycerol using heterogenous catalyst consisting of ion-exchange resin (Lai *et al*., 2006e).

**Table 7** Resin-catalysed oleic acid incorporated DAG and TAG yields and DAG composition from pilot packed-bed reactor production

| Production No. | DAG yield (wt. %) | TAG yield (wt. %) | DAG composition (wt. %)[1] | |
|---|---|---|---|---|
| | | | 13OO | 12OO |
| 1 | 42.9 | 8.1 | 58.6 | 11.1 |
| 2 | 40.3 | 7.7 | 59.4 | 10.5 |
| 3 | 38.4 | 6.1 | 60.1 | 12.3 |
| 4 | 39.2 | 8.5 | 57.1 | 12.7 |
| 5 | 40.5 | 6.6 | 61.1 | 10.6 |

[1]13OO = 1,3-diolein; 12OO = 1,2-diolein. (Source: Lo *et al*., 2007)

**Table 8** Resin-catalysed palmitic and oleic acid incorporated DAG and TAG yields and DAG composition from pilot packed-bed reactor production

| Production No. | DAG yield (wt. %) | TAG yield (wt. %) | DAG composition (wt. %)[1] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 13OO | 12OO | 13PO | 12PO | 13PP | 12PP |
| 1 | 40.5 | 5.1 | 23.5 | 4.4 | 10.5 | 1.5 | 0.5 | 0.1 |
| 2 | 40.2 | 4.6 | 24.5 | 4.0 | 9.9 | 1.2 | 0.5 | 0.1 |
| 3 | 39.1 | 4.6 | 23.2 | 4.1 | 10.0 | 1.1 | 0.6 | 0.1 |
| 4 | 38.2 | 4.4 | 23.1 | 4.0 | 9.5 | 1.0 | 0.5 | 0.1 |
| 5 | 41.8 | 4.8 | 25.0 | 4.4 | 10.4 | 1.4 | 0.5 | 0.1 |

[1] 13OO = 1,3-diolein; 12OO = 1,2-diolein; 13PO = 1-palmitoyl-3-oleoyl-glycerol; 12PO = 1-palmitoyl-2-oleoyl-glycerol; 13PP = 1,3-dipalmitin; 12PP = 1,2-dipalmitin
(Source: Lo *et al*., 2007).

**Table 9** Resin-catalysed stearic and oleic acid incorporated DAG and TAG yields and DAG composition from pilot packed-bed reactor production

| Production No. | DAG yield (wt. %) | TAG yield (wt. %) | DAG composition (wt. %) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 13OO | 12OO | 13SO | 12SO | 13SS | 12SS |
| 1 | 37.5 | 2.6 | 14.4 | 3.4 | 8.5 | 4.1 | 5.7 | 1.5 |
| 2 | 39.2 | 2.5 | 15.3 | 3.8 | 8.9 | 4.2 | 6.0 | 1.6 |
| 3 | 38.3 | 2.2 | 15.2 | 3.6 | 9.0 | 4.1 | 5.6 | 1.7 |
| 4 | 40.1 | 2.5 | 15.2 | 3.4 | 9.5 | 4.4 | 5.9 | 1.5 |
| 5 | 39.8 | 2.3 | 15.3 | 3.4 | 9.0 | 4.4 | 6.5 | 1.4 |

[1] 13OO = 1,3-diolein; 12OO = 1,2-diolein; 13SO = 1-stearoyl-3-oleoyl-glycerol; 12SO = 1-stearoyl-2-oleoyl-glycerol; 13SS = 1,3-distearin; 12SS = 1,2-distearin
(Source: Lo *et al*., 2007).

## HEALTH BENEFITS OF DIACYLGLYCEROL OIL

In the early 2000s, DAG oil received tremendous interest as a replacement for native fats and oils for obesity management (Flickinger and Matsuo, 2003) . The beneficial effects of DAG oil typically lies in the structure of DAG itself, particularly the 1,3-isoform DAG which has metabolic characteristics distinct from TAG (Hideto, 2007). Figure 6 shows the metabolic pathways of DAG and TAG.

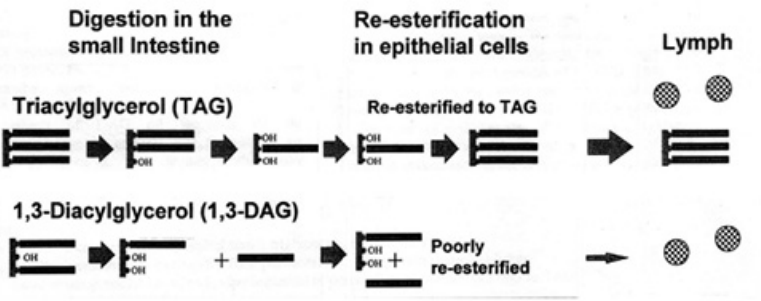


**Figure 6** Metabolic pathway of DAG and TAG
Source: (Matsuo, 2004)

Digestion of DAG was performed by similar gastrointestinal enzymes that hydrolyse TAG. Nevertheless, once digested, DAG does not follow the re-synthetic pathway of TAG, which includes the 2-monoacylglycerol (2-MAG) pathway and the glycerol-3-phosphate (GP) pathway (Friedman and Nylund, 1980). Upon digestion, TAG is hydrolyzed by pancreatic lipase, which is 1,3-specific, into FFA and 2-MAG in the small intestine. 2-MAG is absorbed into small intestine epithelial cells where they are re-esterified into TAG. Subsequently, re-esterified TAG is released into the intestinal lymph and blood circulation as chylomicron, a spherical lipoprotein. Once in the circulatory system, chylomicron is degraded into fatty acids which have the tendency to attach to the inner side of capillary vessels in the heart, muscle and adipose tissue. On the other hand, 1,3-DAG is digested into 1(3)-MAG in the small intestine. As 1(3)- MAG is poorly re-esterified into TAG, release of chylomicron into the intestinal lymph will be lower. Thus, formation of clots at the capillary vessels and fat deposits in the adipose tissue are lower (Matsuo, 2004). This subsequently leads to the reduction of TG-rich lipoprotein, serum TAG and the

postprandial hyperlipidemia, which is contributed by 1,3-DAG particularly (Flickinger and Matsuo, 2003; Rudkowska *et al.*, 2005; Tada, 2004; Yanai *et al.*, 2007).

Further, the fat suppression effect of DAG is mainly contributed by the increases in the rate of β-oxidation upon ingestion of DAG oil (Kamphuis *et al.*, 2003). β-oxidation of fat is the most important element in weight reduction. High level of β-oxidation will suppress accumulation of visceral abdominal fat. The study found that enzymatic activity for β-oxidation in rats' livers increased when rats were fed diets containing 10% of DAG. In coherence, enzymatic activity for fatty acid synthesis in rats' livers decreased upon intake of 10% of DAG (Murase *et al.*, 2001). Another study by Murase *et al.* (2001) on obese mice also showed that high DAG diet increased hepatic acyl-coenzyme Oxidase activity in mice is responsible for oxidizing the fat in hepatic cells. Additionally, β-oxidation and lipid metabolism-related gene expression in C57BL/6J mice was found to be stimulated by the intake of dietary DAG. UPC-2 mRNA was also increased in the small intestine of these obese mice. There was also an elevation in the UPCs expression indicating increased thermogenesis, and therefore, boosting the energy expenditure (Murase *et al.*, 2005).

Several case-control human studies have been conducted to determine the effect of DAG oil consumption on body weight and body fat. Nagao *et al.* (2000) conducted a comparative study on body weight of 38 healthy Japanese men who consumed DAG oil and TAG oil for 16 weeks. They observed significant ($P<0.01$) reductions in body weight (-2.6 ± 0.3 kg), body fat (-22 ± 3.0 $cm^2$) and waist circumference (-4.4 ± 0.6 cm) in the subjects who consumed DAG oil. The Chicago Centre for Clinical Research (CCRC) conducted a parallel double-blind study which involved 127 overweight or obese adults ingesting DAG or TAG diets for a

period of six months. The subjects consuming the DAG diet were found to have significant decrements in body weight and body fat (Matsuo and Tokimitsu, 2001). In another study conducted among 131 overweight subjects in Japan, who consumed 15% of total energy from DAG oil for six months, Maki *et al.* (2002) found significant loss ($P < 0.01$) in terms of body weight and body fat among these overweight subjects. Similarly, Nagao *et al.* (2000) found that DAG oil managed to decrease the total fat, visceral fat area and subcutaneous fat in 38 healthy men with BMI: 21.8 kg/m$^2$ to 27.4 kg/m$^2$ compared to the TAG group, when taken for 16 weeks. In a randomized crossover study, Tada *et al*. (2005) observed that in moderately diabetic subjects postprandial serum TG and lipids was reduced after ingesting emulsified DAG oil or as compared with TAG load.

Much of the literature on clinical and pre-clinical studies conducted on DAG were on long chain unsaturated linoleic/oleic acid-based DAG (Eom *et al.*, 2010; Murase *et al*., 2005; Murase *et al.*, 2002; Murase *et al.*, 2001; Nagao *et al*., 2000; Taguchi *et al.*, 2002). In fact, fatty acid chain length and degree of saturation in the DAG itself may pose varied health effects on the body. Tang *et al*. (2013) found that consumption of both the palm kernel-based diacylglycerol oil, which comprise mainly medium chain fatty acids, and the soy canola- based diacylglycerol have the ability to significantly reduce fat accumulation in the epididymal and retroperitoneal region in C57BL/6N mice, as compared to high-fat diet of 30% palm kernel TG (PKTG) (Table 10 & Figure 7). Compared to TAG, the structural differences in DAG is the main factor contributing to its anti-obesity effect. In a study both the PKDG and SCDG were able to significantly reduce serum glucose, cholesterol, leptin, and insulin levels compared to PKTG (Table 11). The study also showed that both the PKDG and SCDG

reduced the expression of apolipoprotein B mRNA in mice subjects, indicating the potential of PKDG and SCDG in reducing low-density lipoprotein levels in the body. Meanwhile, in terms of gene expression, the expression of acyl-CoA synthase long chain (ACSL) and acyl-CoA synthase medium chain mRNA in the small intestine increased after the ingestion of PKDG. SCDG however induced high expression of ACSL in the liver as well as the small intestine, suggesting that the types of fatty acids that make up DAG may potentially induce β-oxidation in different organs in mice subjects.

**Table 10** Initial body weight, final body weight, energy intake, feed efficiency, fecal lipids in C57BL/6N mice

| | | High Fat diets | | |
|---|---|---|---|---|
| | **Control** | **PKTG** | **PKDG** | **SCDG** |
| Initial body weight (g) | 22.52 ± 1.99[a] | 22.18 ± 2.24[a] | 23.11 ± 0.81[a] | 22.90 ± 1.33[a] |
| Final body weight (g) | 26.99 ± 1.58[b] | 29.68 ± 3.17[a] | 28.06 ± 2.82[ab] | 27.90 ± 3.32[ab] |
| % body weight gain | 19.85 ± 7.56 [c] | 33.83 ± 19.17[a] | 21.43 ± 11.82[b] | 21.68 ± 12.33[b] |
| Energy intake (kcal/cage/day) | 16.05 ± 3.02[b] | 18.23 ± 1.74[a] | 16.87 ± 1.42[ab] | 17.16 ± 1.67[a] |
| Feed efficiency (g/ [kcal/cage/day]) | 0.28 ± 0.02 [b] | 0.41 ± 0.08[a] | 0.29 ± 0.05[b] | 0.29 ± 0.06[b] |
| Fecal lipids (mg/g dried feces) | 27.0 ± 3.21[c] | 47.5 ± 4.81[b] | 47.0 ± 2.87[b] | 50.8 ± 2.98[a] |

Mice were fed with respective diets for 16 weeks. Initial, final body weight and energy intake were determined. Means with the different letters (a and b) are significantly different ($p < 0.05$); control is diet with 5% of PKTG; PK, palm kernel; SC, soy-canola blend; DG, diacylglycerol; TG, triacylglycerol; The feed efficiency was calculated as follows: [body weight gain per cage (g)]/[kcal of food consumed per cage per day]; Values are mean ± SD ($n = 10$). (Adapted from Tang *et al*., 2013)

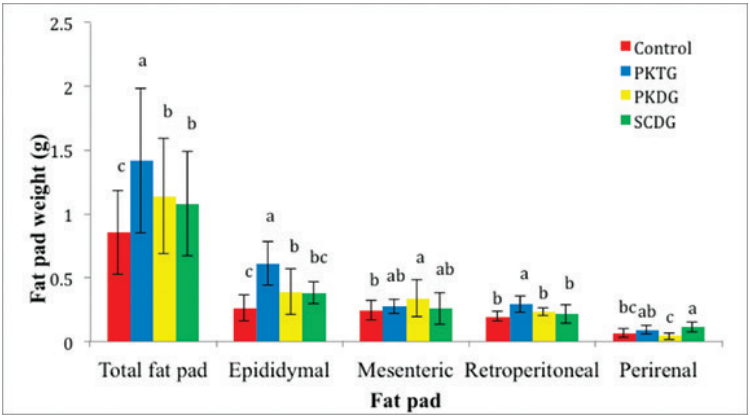


**Figure 7** Fat pad weight in C57BL/6N mice after 16 weeks of feeding with control which is 5% PKTG, PKTG, PKDG and SCDG oil. PKTG; PK, palm kernel; SC, soy-canola blend; DG, diacylglycerol; TG, triacylglycerol; Means with the different letters (a and b) are significantly different ($p < 0.05$); Values are mean ± SD (number of mice, n = 10) (Source: Tang *et al*., 2013)

**Table 11** Fasting serum analysis for C57BL/6N mice fed with control, PKTG , PKDG and SCDG oil

| | Control | High Fat diets | | |
|---|---|---|---|---|
| | | PKTG | PKDG | SCDG |
| Glucose (mg/dl) | 159.56 ± 18.13$^{b}$ | 235.13 ± 41.24$^{a}$ | 179.04 ± 33.74$^{b}$ | 173.29 ± 32.71$^{b}$ |
| Triacylglycerol (mg/dl) | 24.58 ± 15.23$^{b}$ | 33.67 ± 13.87$^{ab}$ | 40.03 ± 20.40$^{a}$ | 36.31 ± 9.29$^{ab}$ |
| Total cholesterol (mg/dl) | 129.18 ± 28.42$^{b}$ | 180.03 ± 30.22$^{a}$ | 143.17 ± 26.55$^{b}$ | 134.28 ± 16.63$^{b}$ |
| LDL(mg/dl) | 99.19 ± 20.05$^{b}$ | 150.46 ± 42.62$^{a}$ | 103.56 ± 24.63$^{b}$ | 124.40 ± 27.68$^{ab}$ |
| HDL(mg/dl) | 36.51 ± 10.68$^{a}$ | 32.62 ± 5.72$^{ab}$ | 28.33 ± 5.07$^{b}$ | 28.41 ± 4.06$^{b}$ |

After 16 weeks feeding trial, blood was collected after 12 h of food deprivation on the final day. Means with the different letters (a and b) are significantly different ($p < 0.05$); control is diet with 5% of PKTG; PKO, palm kernel, SC, Soy-canola blend; DG, diacylglycerol, TG: triacylglycerol; Values are mean ± SD (number of mice, n = 10) (Source: Tang *et al*., 2013)

## APPLICATIONS OF DIACYLGLYCEROL OIL

Since the consumption of DAG oil offers positive effects for weight management (Nagao *et al.*, 2000; Maki *et al.*, 2002; Teramoto *et al.*, 2004), visceral fat, post prandial and fasting triglyceride (Taguchi *et al.*, 2001; Tada *et al.*, 2005), it has prompted great interest among food scientists to incorporate DAG oil into a variety of food products. DAG was authorized, in 2006, as a novel food ingredient for use in cooking oil, fat spreads, salad dressing, mayonnaise, drinks and bakery products. Due to the existence of the hydrophilic OH group in the molecular structure of DAG,

DAG displays distinct physicochemical properties compared to triacylglycerol. Not only will the incorporation of DAG provide the aforementioned health benefits, the DAG structure by itself allows for ease of its applications in food products, particularly for products of water-in-oil or oil-in-water emulsions. The presence of the hydrophilic portion enhances the emulsification and stability of the DAG emulsion allowing it to function alongside monoacylglycerols (MAG) to act as non ionic emulsifiers and stabilizers in the food, cosmetic and pharmaceutical industries, at varied degrees of purity (Nakajima, 2004). The emulsification properties and interfacial phenomena of DAGs were examined by Shimada and Ohashi (2003).

Meat emulsion substituted with DAG was found to be more elastic and solid compared to when lard was used for meat emulsion (Miklos *et al.*, 2011). DAG managed to improve the hydration and binding properties of the meat emulsion. When lard was substituted with 50% and 100% of DAG, the meat emulsion formed gave no fat separation. The total expressible fluid also decreased from 28.2% to 11% when the lard was completely substituted with DAG.

Mayonnaise and salad dressing are oil in water food products where liquid oils are used. Mayonnaise typically contains oil (65% - 80%), egg yolk, vinegar and seasonings. DAG mayonnaise prepared with normal egg yolk is comparable in taste, flavour and colour to the TAG mayonnaise, though the former has slightly higher viscosity. Under accelerated preservation conditions (>40°C), however, DAG mayonnaise stored in laminated tubes and glass container had cracks, causing the aqueous phase to be released into the cracks. However, when phospholipase treated egg yolk was used in the preparation of DAG enriched mayonnaise, no cracks were observed (Kawai, 2004). Phuah *et al.* (2016) made an attempt to substitute the soybean oil in mayonnaise with palm-kernel based

diacylglycerol (PKDG). Figure 8 shows the mayonnaise sample prepared with PKDG and soybean oil. The study found that up to 10% PKDG was suitable to be incorporated in healthy mayonnaise formulation which gave similar rheological and textural properties with the control which consisted of 100% soybean oil (SBO). All the mayonnaise samples exhibited high emulsion stability with a small emulsion droplet size, except for the sample substituted with 20% PKDG. Figure 9 shows the microscopic image of the oil globules in the mayonnaise samples. Replacing up to 15% of PKDG with soybean oil managed to improve the firmness and consistency of the mayonnaise (Table 12).

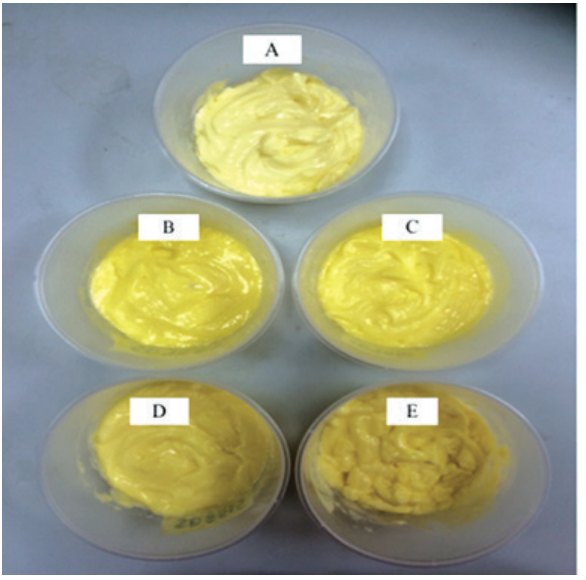


**Figure 8** Mayonnaise samples. A:100% SBO, B: 5% PKDG 95% SBO, C: 10% PKDG 90% SBO, D: 15% PKDG 85% SBO, E: 20% PKDG 80% SBO, where SBO represents soybean oil and PKDG represents palm-kernel based diacylglycerol. (Source: Phuah *et al*., 2016)

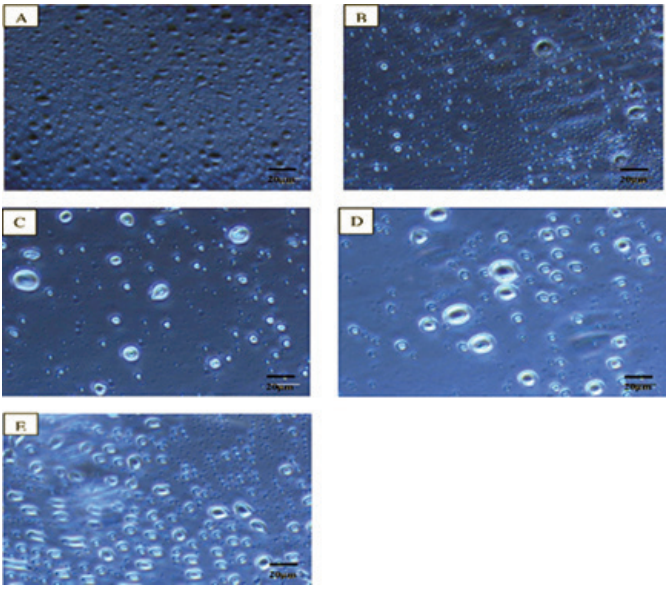


**Figure 9** Microscopic image of oil globules in mayonnaise sample. A:100% SBO, B: 5% PKDG 95% SBO, C:10% PKDG 90% SBO, D:15% PKDG 85% SBO, E: 20% PKDG 80% SBO, where SBO represents soybean oil and PKDG represents palm-kernel based diacylglycerol (Source: Phuah *et al*., 2016)

**Table 12** Texture analysis and rheological properties of mayonnaise samples

| SBO:PKDG | Firmness (g) | Consistency (gsec) | G'G" crossover |
|---|---|---|---|
| 100:0 | $202.8 \pm 3.1^{cd}$ | $1376.8 \pm 17.6^{bc}$ | $250.8 \pm 9.0^{a}$ |
| 95:5 | $246.2 \pm 6.7^{ab}$ | $1813.8 \pm 18.9^{a}$ | $226.5 \pm 12.3^{a}$ |
| 90:10 | $230.9 \pm 3.2^{bc}$ | $1550.5 \pm 74.5^{b}$ | $133.1 \pm 2.2^{c}$ |
| 85:15 | $265.9 \pm 0.5^{a}$ | $1542.7 \pm 41.2^{b}$ | $163.6 \pm 12.8^{b}$ |
| 80:20 | $181.2 \pm 7.8^{d}$ | $1156.8 \pm 32.3^{c}$ | $148.7 \pm 5.9^{bc}$ |

(Source: Phuah *et al*., 2016)

Mori *et al.* (2000) studied the use of DAG oil as frying medium. The resultant fried food which consisted of potatoes, chicken and doughnuts had low water content which prevented it from getting moist easily and reduced its crispiness over a prolonged period of time. Similar to Mori *et al*. (2000) Kudo *et al.* (2005) also reported using DAG oil as a medium for frying or baking potatoes. The potatoes fried or baked in DAG oil had lesser water content, pleasant texture and taste, and higher shelf life. However, at times, frying applications with DAG oil is an issue. This is because DAG tends to have a lower smoke point (30–40°C) than TAG oil with similar fatty acid composition due to its lower molecular weight. Nevertheless, Sakai *et al*. (2006) showed that the stability of DAG can be enhanced with the addition of a fatty acid L-ascorbic ester, catechin, or a natural plant extract, such as rosemary, sage and turmeric extracts, which prevent it from moving towards oxidation besides giving better flavor and appearance. Further study by Sakai *et al*. (2006) revealed that DAG oil can withstand thermal oxidation or hydrolysis during prolonged heating or storage with reduced smoking as well, when the oil composition is used for frying purposes, with the addition of 70 ppm of one or more types of organic carboxylic acids, such as two- to eight-carbon hydroxycarboxylic or dicarboxylic acids and their derivatives.

TAGs consist mainly of medium-chain fatty acids, such as the lauric acid-rich coconut oil which is commonly used as ice cream coating fat. A fascinating study conducted by Cain *et al.* (1999) utilized 50–90% (w/w) DAG and 10–50% (w/w) TAG of vegetable origin as an ice cream coating fat. It was found that this ice cream coating fat resulted in a product that is softer and less brittle but had quicker and smoother meltdown than cocoa butter-based coating fats.

Typical water-in-oil emulsion food includes margarine and fat spreads that are not only consumed at table but also widely used in bakery and confectionery products. The fat/oil content for margarine should be >80% and that of spread, <80%. Plastic fats used in bakery products, such as margarine and shortening, are commonly produced from physical blending of liquid oils with solid fats, hydrogenated or interesterified vegetable oils, which may contain high levels of *trans* fatty acids or saturated fatty acids that are detrimental to health. Substituting the TAG in plastic fats with DAG fat such as palm DAG, partly can thus be an attractive alternative to enhance the overall healthful value and nutritional benefits of the plastic fats.

Stearin fraction of palm-based diacylglycerol when blended with palm mid fraction at the ratio of 50:50 and 60:40 (w/w) were found to be most feasible for the preparation of DAG-enriched shortening (Latip *et al.*, 2013). Further, Cheong *et al.* (2011) also blended the purified palm-based diacylglycerol (PDG) with palm stearin (PS) for application as bakery shortening. The PDG-enriched shortenings with DAG oil content of 50% (DS55), 60% (DS64) and 70% (DS73) and commercial shortening (CS) were assessed for their baking performance in Madeira cakes. It was found that Madeira cakes baked using PDG-enriched shortenings had higher specific volumes ranging from 105% to 108% as compared to Madeira cakes prepared with CS shortening. The enhancement may be due to the emulsifier property of PDG which enhanced air-bubble incorporation and promoted gas bubble stability in the cake batter. Stabilization of the incorporated gas bubble eventually led to the formation of cakes with higher specific volume (Cauvain and Young, 2009). Additionally, sensory evaluation showed that panelists liked all the cakes prepared from different shortenings with overall acceptability range from 6.6 to 6.9 (Figure 10). Substitution

of DAG enriched shortening did not affect the colour, texture, aroma and taste of the Madeira cake.

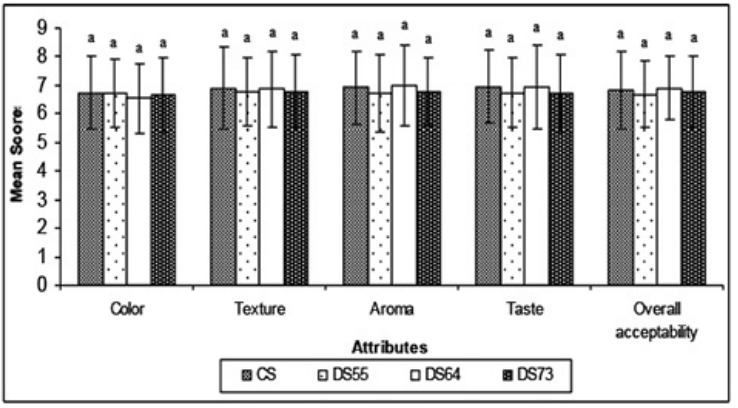


**Figure 10** Mean panelists' ratings for Madeira cakes prepared withdifferent shortenings determined from acceptance tests**.** (PDG-enriched shortening with DAG oil content of 50% (DS55), 60% (DS64) and 70% (DS73) and commercial shortening (CS)
(Source: Cheong et al., 2011)

Additionally, Cheong *et al.* (2010) blended PDG with palm olein for application as potential bakery margarine. The mixture had melting point and SFC profile almost similar to the commercial palm-based bakery margarine. However, production of bakery margarine from these fat systems can be quite costly. In an attempt to reduce the production cost, palm stearin of IV44 was blended into the mixture to produce ternary fat systems suitable for bakery margarine application. These ternary systems were plastic-like fat which was neither solid nor liquid at room temperature. At body temperature, they had low percentages of solids. All the blends crystallized in a mixture of β' and β forms and were composed of small structures. They were also good emulsifying agents.

This PDG enriched margarine was further utilized to prepare Madeira cakes. Trained panelists found the Madeira cakes prepared from PDG-enriched margarine to contain higher moisture content, softer and airier texture than that with CS shortening. The presence of PDG acting as emulsifier reduced the surface tension between the fat and aqueous components, which permitted the fat globules to be fully dispersed into the aqueous component. As such, once the fat component was fully dispersed as small particles in the aqueous system, the water holding capacity of the batter was improved. Additionally, full dispersion of the fat component in the aqueous component also enhanced formation of smaller air cells and improved air incorporation (Sikorski, 2004). Work was also done to produce shelf-stable margarine and soft tub margarine from palm-based DAG. The shelf-stable margarine prepared from the oil blend consisting of palm olein:palm kernel oil: palm-based DAG at the ratio of 42.5:42.5:15 (w/w) had similar solid fat content profile as the commercial shelf stable margarine (Saberi *et al.*, 2011). Additionally, the author also produced soft tub margarine using palm-based DAG where the optimum formulation of sunflower oil: palm kernel olein: palm oil DAG at the ratio of 35:15:50 (w/w) had almost similar profile as the commercial soft tub margarine (Saberi *et al.*, 2012).

Apart from the Madeira cake, PDG enriched margarines consisting of ternary blends of PDG:POo:PS IV44 at 70:25:05 (DOS720), 70:20:10 (DOS721) and 70:15:15 (DOS711) were also incorporated into cookies. The cookies prepared from the different kinds of margarines were well accepted by the panelists. Trained panelists rated all the cookies as having fair acceptability (Figure 11). As for panelists' preference in texture, the panelists showed lower preference for cookies prepared with PDG-enriched margarine as compared to cookies prepared with commercial

margarine CM. This may be due to the softer texture of the cookies prepared with margarines DOS711, DOS720 and DOS721 as compared to the snap texture of the cookies prepared with margarine CM. The aforementioned phenomenon arose due to the higher polarity of the DAG molecule itself which promoted the retention of water during baking thereby increasing gluten development and changing the texture of the cookies from snap type to soft-batch type (Sikorski, 2004). Meanwhile, cookies prepared with margarine CM had the highest panelists' ratings in texture, cohesiveness and color. Sikorski (2004) studied the effect of DAG-enriched plastic fats on the characteristics of cakes, cookies and brownies. He found that although DAG substituted cakes had similar volume as TAG cakes but the former had a softer texture. As for cookies produced with DAG-enriched fats, it was found that they had a variety of textures ranging from snap type to soft-batch type. Meanwhile, there was no significant difference ($p>0.05$) between brownies baked with DAG-enriched fat and TAG fats.

Besides food applications, DAG can also be a potential starting material for various organic products such as phospholipids, glycolipids, lipoprotein, pro-drugs such as 1,3-DAG conjugated chlorambucil for treatment of lymphoma and 1,2-DAG conjugated (S)-(3,4-dihydroxyphenyl)alanine (L-Dopa) for treatment of Parkinson's disease (Garzon-Aburbeh *et al*. 1986; Garzon-Aburbeh *et al.,* 1983; Giacometti *et al.,* 2001; Gonçalves *et al*., 1989).

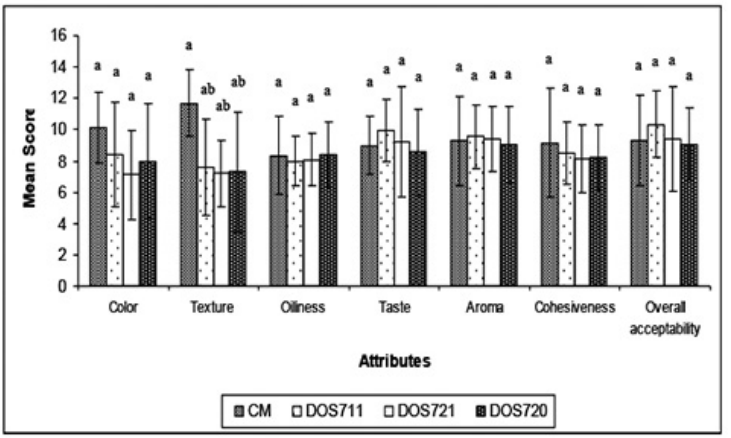


**Figure 11** Mean panelists' ratings of cookies prepared from different margarines determined using qualitative descriptive analysis (QDA). PDG:POo:PS IV44 at 70:25:05 (DOS720), 70:20:10 (DOS721) and 70:15:15 (DOS711) (Source: Cheong *et al*., 2011)

## CONCLUSION

Scientists have been playing around with the structure of fats and oils for many years. With increased understanding about the effects of certain fatty acids, their chain length, degree of unsaturation and positional distribution on the metabolism of TAG or DAG, scientists today are able to construct "healthy fats" which are tailor made to meet specific nutritional or health requirements. The types of fatty acids found in naturally available fats and oils may not be the fatty acid desired by the consumer, nor arranged in the desired stereochemical order in a single TAG/DAG molecule. The option would be to modify the existing fats and oils or to synthesize new ones that have all the desired properties and functions. The modified or newly synthesized lipids may be the answer to solving the love/hate relationship consumers have towards traditional fats.

When Econa cooking oil, which consists of 80% DAG oil, was approved for sales in Japan and US, it was expected that studies by other competitors would be accelerated and newer processing, production and compositions of DAG patents would be filed to challenge the DAG market. This has not happened. The consumers were willing to try the new structured lipids such as DAG. The labelling requirements in several countries were thus revised where food containing DAG was labelled to inform the consumer of potential benefits and health implications. However, the industry was short lived due to the discovery, sometime in 2009-2010, of glycidol fatty acid esters (GEs) as impurities in refined edible oils including DAG oil, which raised concerns of possible exposure to glycidol, a known animal carcinogen, during digestion. There were also studies indicating a correlation between DAG content in oils with the formation of glycidol ester. This, then, is a major challenge to lipid chemists in all fields; to understand the contributions of the underlying composition and structure of oils to the formation of glycidol ester, and to use that understanding to intelligently predict the most advantageous way to mitigate the situation. This issue has affected the outlook for the commercialization of DAG oil. So, until the underlying mechanisms and economical mitigation strategies for removal of glycidol ester in DAG oils is put in place, the potentiality of DAG oils as "Healthy Fats of the Future" remains uncertain.

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## BIOGRAPHY

Lai Oi Ming was born in Kajang, Selangor. She received her First Class Honours Bachelor of Science degree in Biochemistry from Universiti Kebangsaan Malaysia in 1993. She was also awarded the University Gold Medal Award. Upon graduation, she continued her MSc. studies at the same university with Prof. Dr. Salmijah Surif as her supervisor. She completed her MSc. in 2 years with 2 published papers and 3 proceedings. It was at this stage of her life that she felt it would be rewarding to become both an educationist and a researcher. With a scholarship awarded by Universiti Pertanian Malaysia (UPM) in hand, she pursued her PhD degree in 1995 in UPM, under the supervision of Prof. Dr. Hasanah Mohd. Ghazali. She graduated in 1999 with 9 journal articles. This gave her the head start she needed to begin an exciting and challenging career in academia. She was promoted to Associate Professor in 2002 and full Professor in 2012.

Following in the footsteps of her PhD supervisor, she continued her research in the area of enzymatic lipid modification. She is an enthusiast of structured lipids and was invited as a Visiting Research Scientist to the laboratory of Distinguished Research Professor Dr. Casimir Akoh in 2003, at the University of Georgia, USA. Prof. Akoh is one of the most eminent scientists in the area of enzymatic lipid modification and is a prolific author of many papers and books on this subject matter. In 2005, she co-edited a book entitled "Healthful Lipids" published by AOCS Press, USA with Prof. Akoh. Besides being the editor, she also contributed two chapters in the book. Subsequently, she also contributed in 2 other books edited by Prof Akoh called "Handbook of Functional Lipids" (published by CRC Press, USA), and "Food Lipids " (published by Taylor and Francis, USA) as well as another book entitled "Modern Techniques for Food Authentication" (published by Elsevier UK) edited by

Prof. Sun Da-Wen. In 2012, she became the lead editor for the book entitled "Palm oil: Production, Processing, Characterization and Uses" published by AOCS Press, USA. Currently, she also sits on the editorial boards of a number of journals (Food and Bioprocess Technology, PeerJ, Planters and Pertanika Journal of Scholarly Research Reviews).

Oi Ming is actively involved in research. She is the Project Leader for research grants amounting to RM15 million. In 2006, she obtained a total of RM1.8 million worth of research grants from Golden Hope Sdn. Bhd. (now Sime Darby Research Sdn. Bhd) and was awarded the "Excellent Consultant Award" by the University Business Centre, UPM. The collaborative work with Golden Hope produced 4 PhD students sponsored by the company, 10 journal articles, 2 patents filed and granted in Malaysia, US, Japan and Europe, and 7 national and international awards. In 2008, she secured two Technofund projects from the Ministry of Science, Technology and Innovation (MOSTI) amounting to a total of RM10.1 million and completed the building of a fully operational plant for the production of coenzyme $Q_{10}$ API at 99% purity. In the same year, she was awarded 2 Gold Medals during the EUREKA exhibition and research competition held in Brussels, Belgium. She also won the top honours for women by winning the WIPO's (World Intellectual Property Organization) Best Invention by Woman Award for 2008. In 2012, she was the recipient of the Top Research Scientists of Malaysia award from the Academy of Sciences Malaysia.

To date, she has published two joint-edited books, ten book chapters and over 130 scientific articles in peer-reviewed journals, filed 16 patents with nine granted and presented more than 150 papers at various national and international conferences. Her published work has been cited more than 2000 times, and her h-index is 24 as of 1 March 2017.

As a lecturer at the Department of Bioprocess Technology, she has been assigned to teach various undergraduate and postgraduate courses. These courses include enzyme technology, food biotechnology, food biocatalysis, biochemistry, instrumentation, commercialization, entrepreneurship and scientific writing. Oi Ming has supervised 18 PhD and 14 MSc students. Of these, 13 PhD and 10 MSc have since graduated. In addition, she has also been involved in the co-supervision of 18 PhD and 20 MSc students. The supervision of these students has spanned various departments, faculties and universities. Many of these PhD and MSc students eventually became academicians or researchers in Malaysia, Sri Lanka, Singapore, Indonesia, United States, China, United Kingdom and Denmark. Oi Ming also acts as an external examiner for other universities, such as UKM, University of Nottingham Malaysia and Monash University Malaysia. She has also been invited to deliver lectures at conferences and workshops in United States, Netherlands, Vietnam, Thailand, Denmark, China, Canada and Japan.

Apart from teaching and research, Oi Ming is also involved in administrative duties at the national and international levels. She is the Guest Professor at the Department of Food Science and Engineering, College of Science and Engineering, Jinan University, China. She is a member of UPM's Intellectual Property Evaluation Committee, under the purview of the Putra Science Park and also serves as a committee member of the Research Working Committee on Intellectual Property Protection and Licensing, under the purview of RMC, UPM. She was one of the expert panel members for SIRIM's Working Group on Eco-labelling Food Grade Lubricants. In addition, she is also the external assessor for promotion to Associate Professor and Professor position at public and private universities, an assessor of research proposals submitted

to various government agencies and on the Executive Council Panel of Experts for Malaysian Laboratories for Academia-Business Collaboration (MyLAB), Ministry of Higher Education, Malaysia. In the 17th Commonwealth Conference of Education Ministers held in Malaysia, she acted as rapporteur during the Minister's Opening and Closing ceremonies and assisted in drafting of the Vice Chancellor's Mission Statement to the Commonwealth Education Ministers and the Minister's *communiqué* to be brought to CHOGM (Conference of the Head of Governments Meeting) in 2011. As an academician, she has frequently contributed to the scientific community through her role as a regular reviewer of manuscripts submitted to international peer-reviewed journals. Further, she is an active member of several professional bodies and scientific societies. She is happily married to Yee-Ping whom she met in a laboratory in UPM and the couple is blessed with a boy.

## ACKNOWLEDGEMENTS

This inaugural lecture is dedicated to my late father who despite his humble and modest upbringing has always driven into me the importance of education and provided me with important opportunities. Thanks also to my family for their unconditional support for every choice I have made in my life.

I am also greatly indebted to Prof. Dr. Hasanah Mohd. Ghazali, my "guru", who first introduced me to the field of structured lipids. I had a good start in my career because I did my PhD under her supervision. Without her encouragement and guidance, I would not be standing here today. I am also thankful to Prof. Dr. Tan Chin Ping and Dr Kamariah Long for their long friendship, support, advice, jokes and constant encouragement during the more difficult times of my career. They were always there for me in the good and the bad times. I must also mention Prof. Datin Paduka Dr. Khatijah Yusoff, for always encouraging and nurturing my ideas. She has always been supportive of all my endeavours. Further, to old friends who became business partners, En. Hishamuddin Mohamed and Ms. Leow Min Min, thank you for teaching me the intricacies of business. To Mr. Stanley Tan, General Manager of Shimadzu Malaysia Sdn. Bhd., your years of friendship and continuous support mean a lot to me. Special gratitude also goes to all my Deans of the Faculty of Biotechnology & Biomolecular Sciences and those from the former Faculty of Food Science and Biotechnology and Heads of Department of Bioprocess Technology, past and present, all colleagues, academic and non-academic staff, for their continuous support and cooperation. I bow and doff my cap to all of you.

I would also like to take this opportunity to thank all my postgraduate students who are too many to mention here. They bore the brunt of my career development and I would like to acknowledge their contributions. They helped propel me to where I am today.

Having spent 18 years of my working life in Universiti Putra Malaysia, I would like to express my gratitude to UPM for providing me with a conducive environment to excel. I would also like to express my sincere gratitude to all funding agencies for their support in pursuing my research. Special mention also goes to Tuan Haji Khairudin Hashim, Dr. Mohd. Suria Affandi Yusoff, Dr Razam Abdul Latip and Mr. Ahmadilfitri Noor of Sime Darby Research Sdn. Bhd. for the grants to pursue and complete the project on palm-based diacylglycerol.

Isaac Newton once said that "*If I have seen further than others, it is by standing upon the shoulders of giants*." This has never rang more true than at this very moment. I have met so many people who have motivated, inspired and helped me along the way. The journey that I had taken would not have been as meaningful and enjoyable without all of you. It is not possible for me to thank each and every one of you, but please know that your kindness and support will always be remembered and treasured.

Finally, thank you to my husband, Yee-Ping, and son, Min Ho, who listened to me ramble about crazy ideas, watched my back when I needed it most, forgave me for my shortcomings and pulled me back on course when I strayed unmoored. I love you both!

Lai Oi Ming

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